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## GC-MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF DEVELOPMENTAL TOXIC EFFECTS ON PERCUTANEOUS EXPOSURE OF RATS TO VX

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14. ABSTRACT: This report documents the results of gas chromatography–tandem mass spectrometry (GC-MS/MS) analyses of blood, tissues, and organs (heart, lung, liver, kidney, brain, eye, diaphragm, and skin) that were obtained from rats (postnatal days 42 and 70) percutaneously exposed to various concentrations of <i>O</i> -ethyl <i>S</i> -(2-diisopropylaminoethyl) methylphosphonothioate (VX). The amount of <i>O</i> -ethyl methylphosphonofluoridate (VX-G) present in these biological samples was quantified using chemical ionization mass spectrometry with isotope dilution. In addition, free VX present on the skin surface was quantified from cellophane tape strip sampling using liquid chromatography–tandem mass spectrometry (LC-MS/MS).													
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## **PREFACE**

The work described in this report was authorized under project no. BARDA CBRN OS 75442 ASPR-11-04601 and U.S. Army Medical Research Institute of Chemical Defense Protocol 1-12-U-1001. The work was started in April 2013 and completed in July 2013, as recorded in ECBC notebook 13-0001.

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This report has been approved for public release.

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# GC-MS/MS ANALYSES OF BIOLOGICAL SAMPLES IN SUPPORT OF DEVELOPMENTAL TOXIC EFFECTS ON PERCUTANEOUS EXPOSURE OF RATS TO VX

## 1. INTRODUCTION

Chemical warfare nerve agents (CWNAs) include G-agents such as tabun (ethyl *N,N*-dimethylphosphoramidocyanidate; GA), sarin (isopropyl methylphosphonofluoridate; GB), soman (pinacolyl methylphosphonofluoridate; GD), and cyclosarin (cyclohexyl methylphosphonofluoridate; GF), as well as less-volatile V-agents such as VX (*O*-ethyl *S*-[2-diisopropylaminoethyl] methylphosphonothioate) and Russian VX (*O*-isobutyl *S*-[(2-diethylamino)ethyl] methylphosphonothioate). Like organophosphorus pesticides, CWNAs exert their toxicological effects by inhibiting acetylcholinesterase (AChE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine (ACh) in the central and peripheral nervous systems. ACh accumulation within the synaptic cleft after extensive AChE inhibition increases and prolongs the stimulation of muscarinic and nicotinic receptors on autonomic ganglia, end-organs, myocytes, and postsynaptic neurons, which leads to an acute cholinergic crisis characterized by autonomic and cardiac dysfunction, involuntary movements, miosis, muscle fasciculations, respiratory distress, and seizures (Russell and Overstreet, 1987).

In the event of a mass casualty situation involving the release of CWNA amongst a civilian population or the military community, pregnant women, infants, and small children are likely to be exposed. For example, four pregnant women between 9 and 36 weeks of gestation were admitted to the hospital with mild cholinergic symptoms after the release of GB in the Tokyo subway system (Ohbu et al., 1997). In fact, infants and small children may be at greater risk for inhalation exposure to CWNAs than adults because of their greater minute ventilation rates (Bloomfield, 2002) and larger surface-area-to-body-mass ratios (Guzelian et al., 1992), respectively. In addition, infants have thinner, less acidic, and more hydrated strata cornea than adults, with similar or higher transepidermal water loss (TEWL) values depending on the anatomic location (Stamatas et al., 2011). This suggests that CWNAs may penetrate the skin of infants and children more easily. Infants and small children are also considered to be at greater risk for seizures relative to adults (Ben-Ari and Holmes, 2006), and early-life seizures increase the likelihood of seizure-induced brain damage in adulthood (Thompson and Wasterlain, 1997).

Unfortunately, the majority of research on the toxic effects of nerve agent exposure has been focused on combat soldiers, who are typically characterized as males between 18 and 25 years old. Federal agencies have little data to draw from when making recommendations for pediatric doses of medical countermeasures (Baker, 2007), which may lead to inadequate treatment or even overdose.

Therefore, the report *Developmental Toxic Effects on Whole-Body and Percutaneous Exposure to Chemical Warfare Nerve Agents (CWNA) in Rats: Effects on Brain and Behavior*, from the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD; 2012), provides additional data needed to develop a pediatric animal model of nerve agent exposure to make more accurate human risk assessments. In addition,

physiologically based pharmacokinetic and pharmacodynamic modeling, which was incorporated into the study, yields a quantitative basis for extrapolating animal-to-human exposure conditions and predicting the human response to a chemical of interest. Earlier reports (McGuire et al., 2015a,b) documented the results of the gas chromatography-tandem mass spectrometry (GC-MS/MS) analyses of blood, tissues, and organs (heart, lung, liver, kidney, brain, eye, and diaphragm) that were used to quantify the amounts of free and regenerated GB present in these biological samples from whole-body and subcutaneous exposures. This report details the results of the GC-MS/MS analyses of blood, tissues, and organs to quantify the amounts of *O*-ethyl methylphosphonofluoridate (VX-G) present from percutaneous exposures to VX. Also, amounts of residual VX present on skin tissues were determined from cellophane tape strip samplings using liquid chromatography–tandem mass spectrometry (LC-MS/MS). These biosamples were collected from male and female rats that had been percutaneously exposed to VX at two different stages of development (puberty and early adulthood) to evaluate age-related differences in the lethal potency of VX using an operationally relevant route of exposure (Wright et al., 2016). Compared with adults, pubescent children have drier skin, with similar or higher TEWL values, depending on the anatomic location (Akutsu et al., 2009), and the surge of hormones during puberty triggers a rapid increase in sebum production (Leung et al., 2013).

## 2. METHODS

### 2.1 Animal Exposures

Male and female Sprague-Dawley rats (CD IGS rats) were purchased from Charles River Laboratories (Kingston, NY) and divided into two groups based on their age (postnatal days [PNDs] 42 and 70). Table 1 shows the pre-exposure body weights in grams (mean  $\pm$  standard deviation) for each age group and gender along with the total number (*N*) of rats per group. The rats were individually housed in temperature- and humidity-controlled colony rooms ( $21 \pm 2$  °C and  $60 \pm 20\%$ , respectively) under a 12 h light/dark cycle (lights on at 0600). Food and water were available ad libitum.

Table 1. Rat Body Weight before Exposure

Group	Male		Female	
	Weight (g)	<i>N</i>	Weight (g)	<i>N</i>
PND 42	231 $\pm$ 14	26	170 $\pm$ 7	24
PND 70	353 $\pm$ 13	23	232 $\pm$ 12	24

*N*, number of rats.

Approximately 24 h prior to the exposures, the fur on the right flank of each rat was clipped using an Oster A5 clipper and a no. 40 CryogenX blade (Sunbeam Products; Boca Raton, FL). Care was taken to limit razor burn, and VX was not applied to areas with visible abrasions. On the morning of the exposures, the rats were moved to the procedure room, which contained a bank of chemical fume hoods. A circle was drawn on the clipped flank of each rat

with a black permanent marker. Each rat was then fitted with an Elizabethan collar, placed in a polycarbonate cage, and moved into one of the hoods, where it remained for the rest of the study.

VX was obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC) chemical agent standard analytical reagent material (CASARM) stock. A digital 0.5  $\mu\text{L}$  syringe (Hamilton Laboratory Products; Reno, NV) was used to apply the VX, in its neat (undiluted) form, to the center of the circle drawn on each unanesthetized rat. The volume of VX applied to each rat ranged from 2.5 to 115 nL, and the specific gravity of VX (1.01 g/mL) along with each rat's body weight was used to calculate the dose that was administered. Exposures were conducted between 0900 and 1100, and toxic signs were continuously monitored throughout the business day. Biosamples (brain, diaphragm, eye, heart, lung, liver, and kidney) were collected at time of death or 48 h post-exposure for survivors.

All percutaneous exposures were conducted by USAMRICD personnel. All biosamples were snap-frozen in liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$  until analysis. For the percutaneous exposures, a skin sample from the exposure site, as well as an adjacent, unexposed site, was collected. In addition, three cellophane tape strips were collected from the percutaneous exposure site for free VX analysis.

## **2.2 Sample Preparation and Analysis**

### **2.2.1 Chemical Materials**

EA 1207 (VX-G) and deuterated ( $^2\text{H}_5$ ) EA 1207 were obtained from ECBC stock. Before use, the EA 1207 was verified by quantitative  $^{31}\text{P}$  NMR spectrometry as  $78.43 \pm 0.56\text{ wt \%}$  (laboratory notebook [NB] 11-0003-114), and the  $^2\text{H}_5$ -EA 1207 was verified as  $68.81 \pm 0.9\text{ wt \%}$  (NB 11-0003-115). VX (VX-U-1244-CTF-N) and  $^2\text{H}_5$ -VX were obtained from ECBC CASARM stock. Before use, the CASARM-grade VX was verified as  $95.8\text{ wt \%}$  (NB 11-0003-97). A purity determination was not performed on the  $^2\text{H}_5$ -VX. Potassium fluoride (KF), 2-propanol (IPA), ethyl acetate, glacial acetic acid, and anhydrous sodium sulfate were obtained from Sigma-Aldrich (St. Louis, MO) at  $\geq 99\%$  purity. Sodium acetate was purchased from Fischer Chemicals (Fair Lawn, NJ) at  $>99\%$  purity. Ammonia and methane were obtained from Sigma-Aldrich, and helium was obtained from Messer (Malvern, PA); all were at  $>99.9\%$  purity.

### **2.2.2 Stock Solutions and Calibration Standards**

Stock solutions of VX-G and  $^2\text{H}_5$ -VX-G (internal standard, IS) were prepared in IPA at concentrations of 1.524 mg/mL (NB 11-0003-110-01) and 1.473 mg/mL (NB 11-0003-111-01), respectively, and stored at  $-20\text{ }^{\circ}\text{C}$  until use. Working solutions (5–10  $\mu\text{g/mL}$ ) were prepared by diluting the stock solutions in ethyl acetate. Calibration standards of VX-G were prepared by diluting the working solution to obtain the following 12 concentration points: 0.5, 1, 5, 10, 25, 50, 100, 200, 400, 600, 800, and 1000 ng/mL (NBs 11-0003-117-04 through 11-0003-117-15). Each calibration standard also contained 200 ng/mL  $^2\text{H}_5$ -VX-G diluted from the working solution. All calibration standards were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis.

Stock solutions of VX and  $^2\text{H}_5\text{-VX}$  (IS) were prepared in IPA at concentrations of 1.72 mg/mL (NB 11-0003-97-01) and 1.909 mg/mL (NB 11-0003-98-01), respectively, and stored at  $-20\text{ }^\circ\text{C}$  until use. Working solutions (2.5–10  $\mu\text{g/mL}$ ) were prepared by diluting the stock solutions in IPA. Calibration standards of VX were prepared by diluting the working solution to obtain the following 12 concentration points: 0.5, 1, 5, 10, 25, 50, 100, 200, 400, 600, 800, and 1000 ng/mL (NBs 11-0003-99-06 through 11-0003-99-17). Each calibration standard also contained 50 ng/mL  $^2\text{H}_5\text{-VX}$  diluted from the working solution. All calibration standards were stored at  $-20\text{ }^\circ\text{C}$  until analysis.

For the VX-G and VX assays, calibration curves were constructed using the 12 calibration standards from the respective analytes, where *Relative Response* (defined as  $\text{Area}_{\text{Analyte}}/\text{Area}_{\text{IS}}$ ) was plotted against *Relative Concentration* (defined as  $\text{Concentration (ng/mL)}_{\text{Analyte}}/\text{Concentration (ng/mL)}_{\text{IS}}$ ). For the VX-G calibration curve, a quadratic curve fit was used with a  $1/x$  weighting factor, and for the VX calibration curve, a linear curve fit was used, also with a  $1/x$  weighting factor. Typically, these calibration curves yield correlations of  $R^2 = 0.999$  over 3 orders of magnitude, where  $R^2$  is defined as the coefficient of determination.

### 2.2.3 Analytical Method

VX-G sample assays were performed using an Agilent 7000A Triple Quad GC/MS instrument (Agilent Technologies; Santa Clara, CA). Gas chromatographic separations were achieved using an RTX-1701 column (30 m  $\times$  0.25 mm i.d., 0–25  $\mu\text{m}$  film thickness; Restek Corporation; Bellefonte, PA). The carrier gas was helium, with a flow rate of 1 mL/min. Injections of 2.0 or 3.0  $\mu\text{L}$  were made using an Agilent 7693 ALS autoinjector into a splitless injector port at a temperature of  $225\text{ }^\circ\text{C}$ . The initial oven temperature of  $35\text{ }^\circ\text{C}$  was held for 6 s, then ramped to  $100\text{ }^\circ\text{C}$  at  $15\text{ }^\circ\text{C/min}$ , and ramped again at  $35\text{ }^\circ\text{C/min}$  to  $175\text{ }^\circ\text{C}$ . After each analysis was complete, the column was backflushed at  $280\text{ }^\circ\text{C}$  for 4 min at reduced inlet pressure ( $-6.3\text{ mL/min}$ ). Typical retention times for VX-G and its deuterated standard are 5.5 min.

Samples were ionized by positive-ion chemical ionization (CI) with ammonia reagent gas. CI source conditions were optimized using Fluoroether E3 (Chemical Abstracts Service [CAS] registry number 3330-16-3; Agilent Technologies) tuning compound with methane reagent gas. Mass spectra were obtained at a dwell time of 0.2 s for each transition in the multiple reaction monitoring (MRM) mode. Helium was used as the collision gas with a collision energy (CE) of 12 V. The CE was optimized for the mass-to-charge ratio ( $m/z$ )  $144 > 99$  transition for VX-G and the  $m/z$   $149 > 100$  transition for  $^2\text{H}_5\text{-VX-G}$ . The MassHunter software provided with the Agilent 7000A system was used to process and analyze the data. The software provides automated peak detection, calibration, and quantitation.

VX sample assays were performed using an Agilent 6410 triple quadrupole LC/MS instrument (Agilent Technologies). Liquid chromatographic separations were achieved using an Agilent Zorbax Eclipse XDB-C18 column (4.6  $\times$  150 mm, 5  $\mu\text{m}$ ) maintained at  $40\text{ }^\circ\text{C}$  and a flow rate of 1 mL/min. A 1  $\mu\text{L}$  injection was analyzed with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in methanol) as follows: hold 0.1% B for 2 min, 0.1–95% B in 5 min, hold 1 min, 95–0.1% B in 3 min, and hold 2 min for re-equilibration. Typical retention times for VX and its deuterated standard are 6.7 min.

Samples were ionized by electrospray ionization (ESI) in positive-ion mode. ESI source conditions were optimized using an electrospray tuning mix (part number G2421-60001; Agilent Technologies). Mass spectra were obtained at a dwell time of 0.2 s for each transition in the MRM mode. Nitrogen was used as the collision gas with a CE of 20 V. For VX, the CE was optimized for the  $m/z$  268 > 128 transition as the quantitation transition, and the  $m/z$  268 > 86 transition was optimized for the confirmation transition. For  $^2\text{H}_5\text{-VX}$ , the CE was optimized for the  $m/z$  273 > 128 transition as the quantitation transition, and the  $m/z$  273 > 86 transition was optimized for the confirmation transition. The MassHunter software provided with the Agilent 6410 system was used to process and analyze the data.

#### 2.2.4 Sample Preparation

Sample preparations for this study were similar to those published by McGuire et al. (2015a,b). Upon arrival, all biological samples were stored at  $-80^\circ\text{C}$  until analysis. Whole blood samples were extracted for VX-G using Oasis HLB 30  $\mu\text{m}$  solid-phase extraction (SPE) cartridges (Waters Corporation; Milford, MA), which were first conditioned with 1 mL each of ethyl acetate, IPA, and pH 4.0 acetate buffer (0.01 M sodium acetate and 0.2 M glacial acetic acid). After the sample of blood in a 2.0 mL microcentrifuge tube (Sigma-Aldrich) was weighed, 1 mL of acetate buffer, 200  $\mu\text{L}$  of KF solution (6 M), and 1  $\mu\text{L}$  of IS,  $^2\text{H}_5\text{-VX-G}$ , were added. The mixture was vortexed for 10–20 s and then centrifuged at 15,000 rpm for 5 min using a Micromax microcentrifuge (Thermo IEC; Needham Heights, MA). The supernatant liquid was transferred to the SPE cartridge, and the sediment at the bottom of the microcentrifuge tube was resuspended with 750  $\mu\text{L}$  of acetate buffer and 200  $\mu\text{L}$  of KF solution. This mixture was also vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. After the mixture was added to the SPE cartridge, it was allowed to drain under a gentle vacuum. The analytes were eluted with 1 mL of ethyl acetate, which was collected and dried over anhydrous sodium sulfate. The ethyl acetate was withdrawn from the collection tube, filtered through a 0.2  $\mu\text{m}$  nylon Acrodisc syringe filter (Pall Gelman Laboratory; Ann Arbor, MI) into a GC autosampler vial (Agilent Technologies), and then concentrated to 50  $\mu\text{L}$  for analysis.

Tissue and organ sample extracts were prepared in a similar manner; freeze-fracture pulverization under cryogenic temperatures was performed before SPE extraction. A CryoPrep system (Covaris; Woburn, MA) was used to pulverize 0.5–1 g of tissue. The pulverized sample was mixed with 1 mL of acetate buffer, 200  $\mu\text{L}$  of KF solution, and 1  $\mu\text{L}$  of IS. This sample was then subjected to focused acoustics using an S-series focused ultrasonicator. This system directs precisely controlled cavitation and acoustic streaming to a focal point within a sample-treatment vessel in a noncontact, isothermal process. After centrifugation at 4500 rpm for 15 min using a Sorvall Legend X1R centrifuge (Thermo Fisher Scientific; Waltham, MA), the supernatant liquid was transferred to the SPE cartridge, and the sediment at the bottom of the sample tube was resuspended with 750  $\mu\text{L}$  of acetate buffer and 200  $\mu\text{L}$  of KF solution. This mixture was vortex-mixed and centrifuged, and the resulting liquid was added to the original solution. Further sample processing was performed in a manner that was identical to that of the blood samples.

VX assays of the cellophane tape strips were prepared by adding 5 mL of IPA to the sample in a 20 mL scintillation vial (Wheaton; Millville, NJ) and sonicating the solution for 20 min. A 49  $\mu$ L aliquot was removed, and 1  $\mu$ L of IS,  $^2\text{H}_5\text{-VX}$ , was added.

### **3. RESULTS AND DISCUSSION**

#### **3.1 VX-G Assays**

The following results have been recorded in ECBC NB 13-0001. Table 2 summarizes the data from the VX-G assays of whole blood and various tissues and organs that were obtained from PND 42 rats after percutaneous exposure to various doses of VX. Table 3 shows similar results from the percutaneous exposures for the additional age group.

Although a detailed analysis of the pharmacokinetics of VX absorption, distribution, metabolism, and elimination in rats is beyond the scope of this report, several observations were made. Plots of the VX-G concentrations (in nanograms per gram) from the exposed skin samples against the doses (in micrograms per kilogram) administered percutaneously yielded a dose-response curve for PND 42 female rats that was noticeably different from those for the PND 70 female rats and both the PND 42 and PND 70 male rats. The difference may be indicative of a lower susceptibility of the pubescent female rats to the effects of percutaneous VX exposure.

All of the unexposed skin samples that were harvested and analyzed showed the presence of VX-G, indicating systemic exposure to VX. The systemic exposure was also indicated by the presence of VX-G in the various tissues that were analyzed.

#### **3.2 VX Assays**

The following results were recorded in ECBC NB 13-0001. Table 4 summarizes the data from the VX assays of the extracts from cellophane tapes obtained from PND 42 rats after percutaneous exposure to various doses of VX. Table 5 shows similar results from the percutaneous exposures for the additional age group. An examination of the total amounts of VX identified from the individual cellophane tape strips appears to show some measure of protection in the PND 42 female rats. Linear plots of the total VX concentrations (in nanograms) from the exposed skin sample tapes against the doses (in micrograms per kilogram) administered percutaneously yielded curves that imply less VX was on the skin for a similar percutaneous exposure of PND 42 female rats, as compared with PND 70 female rats and both the PND 42 and PND 70 male rats.

Because the goal of this report is to document the results of the GC-MS/MS and LC-MS/MS analyses, no further interpretations were attempted. All of the data have been transferred to Dr. Jeffery M. Gearhart of the Henry M. Jackson Foundation for the Advancement of Military Medicine at Wright-Patterson Air Force Base, for further efforts to develop the appropriate physiologically based pharmacokinetic and pharmacodynamic models to predict the biological impact of VX exposure in young animals.

Table 2. Results from VX-G Assays of PND 42 Rats

Rat No.	Sex	Dose (µg/kg)	VX-G (ng/g)								Exp. Skin	Unexp. Skin
			Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm		
1685	F	315	NS								55,884	NS
1686	F	44									2913	3.4458
1687	F	183									12,850	NS
1688	F	315									34,856	NS
1689	F	44									218.8245	1.8217
1690	M	115									13,952	NS
1691	M	190									24,991	NS
1692	M	21	0.1092	BDL	0.1337	0.0718	0.0930	0.3986	BDL	BDL	177.1640	*
1693	M	72	0.9676	NS							6,931	34.4880
1694	M	315	NS								25,858	NS
1695	F	186									16,268	NS
1696	F	81									13,429	16.3417
1697	F	31									11.5106	*
1698	F	74									8,616	2.3423
1699	F	100									19,548	2.2913
1700	M	21									0.0851	BDL
1701	M	311	NS								41,036	NS
1702	M	187									39,966	NS
1703	M	54	NS	0.4935	0.5515	0.3211	0.4322	2.2262	0.4549	0.1160	7352	2.2492
1704	M	31	NS	1.1547	0.5207	0.4972	NS	1.0884	0.1577	3.0531	732.7168	3.2517
1706	F	100	NS								26,615	6.2112
1707	F	74									12,681	2.8647
1709	F	186									14,556	NS
1710	M	184									29,243	NS
1711	M	73	NS	0.1615	0.3938	0.2536	0.2986	2.4459	0.5578	0.2380	18,316	4.2183
1712	M	53	NS	0.2259	0.6195	0.3020	0.8225	2.4521	BDL	0.3172	8,811	1.9538
1713	M	114	NS								11,785	NS

\*These samples were lost during assay preparation.

BDL, below detection limit (<0.05 ng/g).

NS, no sample was received.

Exp., exposed.

Unexp., unexposed.

Table 2. Results from VX-G Assays of PND 42 Rats (Continued)

Rat No.	Sex	Dose (µg/kg)	VX-G (ng/g)								Exp. Skin	Unexp. Skin
			Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm		
1714	M	31	NS								733.5326	1.3947
1951	F	75									36.2201	0.1777
1952	F	63									3,124	0.7835
1953	F	77									233.9409	1.1129
1954	F	59									51.4386	0.4335
1955	F	60									176.4109	0.3589
1956	F	48									98.4650	1.1791
1957	F	46									148.3219	0.5823
1958	F	58									182.5693	1.6875
1959	F	86									3,781	1.1794
1960	M	21	0.2548	0.1107	0.0855	0.1615	0.8205	0.3282	BDL	BDL	12.6588	0.1331
1961	M	23	0.2786	0.0674	0.1133	0.2367	0.8428	0.5391	BDL	BDL	20.8657	0.1674
1962	M	23	0.3570	0.0549	0.0686	0.1099	0.5832	0.4492	BDL	BDL	880.2990	0.1060
1963	M	57	1.3507	0.1083	0.5023	0.3295	0.3199	2.3079	0.6660	0.1715	3,384	4.4796
1964	M	35	0.3358	0.0608	0.1899	0.6437	1.3452	0.7878	0.1922	BDL	27.4884	0.3426
1965	M	57	NS	0.2527	0.4537	0.2594	0.4645	2.6529	2.6945	0.2864	2,316	21.1182
1966	M	55	1.1182	0.2350	0.3425	0.3784	0.9879	2.0786	BDL	0.1705	2,154	0.8628
1967	M	14	0.1962	BDL	0.0623	0.1324	0.3769	0.1859	BDL	BDL	49.0986	0.1178
1968	M	23	0.3724	0.1138	0.1934	0.3738	1.0252	0.6835	0.1328	BDL	57.4234	0.2689
1969	M	45	0.2831	0.1284	0.1451	0.3851	0.9422	0.5725	0.0528	BDL	144.0878	0.1873
1970	M	44	NS	0.0853	0.2154	0.5735	1.2952	0.8518	0.0825	BDL	188.1556	0.2710

BDL, below detection limit (&lt;0.05 ng/g).

NS, no sample was received.

Exp., exposed.

Unexp., unexposed.

Table 3. Results from VX-G Assays of PND 70 Rats

Rat No.	Sex	Dose (µg/kg)	VX-G (ng/g)								Exp. Skin	Unexp. Skin
			Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm		
1715	F	78	NS								10,989	6.6768
1716	F	23									106.4012	*
1717	F	75									2,194	6.6526
1718	F	190									20,364	NS
1720	M	114									5,352	4.9114
1722	M	312									32,442	NS
1723	M	48	0.8379	0.3170	0.6068	0.2668	0.5369	3.0601	0.5421	0.2423	2,798	15.2082
1725	F	115	NS								11,611	NS
1726	F	308									22,171	NS
1728	F	33									762.6475	2.2562
1729	F	57									558.1760	4.9613
1730	M	35	0.2031	0.1464	0.2498	0.2831	0.2735	0.8995	0.1904	0.2522	632.6992	3.9571
1731	M	314	NS								56,238	NS
1732	M	190									26,728	NS
1733	M	21	0.1275	0.0594	0.1441	0.2061	0.1883	0.4287	BDL	BDL	140.1091	*
1734	M	21	0.1184	BDL	0.1562	0.1213	0.1716	0.5546	BDL	BDL	85.1259	*
1735	F	33	NS								533.0036	*
1737	F	57									2,379	1.7024
1738	F	56									1,611	*
1740	M	187									22,375	NS
1741	M	48									1,012	*
1742	M	35	0.1873	0.1678	0.2456	0.4175	0.1858	1.0036	0.1073	BDL	479.2745	*
1743	M	14	0.0874	BDL	0.0988	0.0919	0.1069	0.4926	BDL	BDL	39.4648	0.3523
1744	M	110	NS								8,704	NS
1855	F	51									311.4882	0.6858
1856	F	54									8,209	2.4669
1857	F	86									10,930	48.0940

\*These samples were lost during assay preparation.

BDL, below detection limit (<0.05 ng/g).

NS, no sample was received.

Exp., exposed.

Unexp., unexposed.

Table 3. Results from VX-G Assays of PND 70 Rats (Continued)

Rat No.	Sex	Dose (µg/kg)	VX-G (ng/g)								Exp. Skin	Unexp. Skin
			Whole Blood	Heart	Lung	Liver	Kidney	Brain	Eye	Diaphragm		
1858	F	93	NS								11,660	5.8294
1859	F	43									2,066	NS
1860	F	87									18,351	9.2706
1861	F	46									4,670	7.1044
1862	F	56									6,964	7.9033
1863	F	43									1,525	0.8364
1864	M	73									2,852	1.8977
1865	M	38	0.3927	0.1730	0.5777	0.4362	0.4811	2.5019	0.3050	0.1654	4,281	13.5121
1866	M	45	NS								1,225	1.7436
1867	M	73	0.6743	0.4168	0.5372	0.4317	0.8378	1.9525	0.4065	0.1512	2,637	5.4971
1868	M	37	0.1883	0.0789	0.1682	0.3474	0.2418	0.7271	BDL	BDL	146.3189	0.7247
1869	M	75	0.8735	0.1561	0.5913	0.2340	0.5600	2.0384	0.5586	0.3543	4,741	1.6825
1870	M	51	0.6646	0.1926	0.5115	0.4792	0.3902	2.0993	0.2074	0.2293	4,774	3.4218
1871	M	51	0.6239	0.1621	0.5596	0.5425	0.3276	1.8638	0.3189	0.1320	7,021	1.5943
1872	M	43	0.1828	0.2103	0.4174	0.2564	0.4346	1.4826	0.1602	BDL	677.4627	0.8765

BDL, below detection limit (&lt;0.05 ng/g).

NS, no sample was received.

Exp., exposed.

Unexp., unexposed.

Table 4. Results from VX Cellophane Tape Strip Assays of PND 42 Rats

Rat No.	Sex	Dose ( $\mu\text{g/kg}$ )	VX from Tape Strip (ng)			Total VX (ng)
			A	B	C	
1685	F	315	218.5000	252.9851	75.1881	546.6732
1686	F	44	0.6255	0.9535	0.3840	1.9630
1687	F	183	48.8229	51.8111	49.1029	149.7369
1688	F	315	211.2247	68.5948	63.3335	343.1530
1689	F	44	0.5570	1.3750	1.1275	3.0595
1690	M	115	185.3266	14.7983	6.8604	206.9852
1691	M	190	352.7801	51.5780	32.6431	437.0012
1692	M	21	9.5855	8.3230	7.0510	24.9595
1693	M	72	29.0563	49.0809	27.3260	105.4632
1694	M	315	568.6548	400.3847	63.7798	1032.8193
1695	F	186	87.7182	51.9288	28.9247	168.5718
1696	F	81	55.2405	29.2843	32.2936	116.8184
1697	F	31	2.3155	2.1845	2.2945	6.7945
1698	F	74	59.9226	83.9001	32.0435	175.8662
1699	F	100	34.0653	23.2597	17.3728	74.6979
1700	M	21	2.3245	2.3100	2.1895	6.8240
1701	M	311	25.8487	5.1104	17.5255	48.4847
1702	M	187	137.6617	204.2132	62.8536	404.7285
1703	M	54	87.3904	43.9445	42.2771	173.6120
1704	M	31	15.5945	19.3825	30.7725	65.7495
1706	F	100	83.7096	80.9183	235.6062	400.2341
1707	F	74	52.8014	63.2311	44.7680	160.8004
1709	F	186	41.4315	63.3927	23.3215	128.1458
1710	M	184	262.4849	216.2989	242.2704	721.0542
1711	M	73	209.8973	77.8005	75.2949	362.9926
1712	M	53	232.0005	151.3298	105.7316	489.0619
1713	M	114	224.9899	137.7858	75.2538	438.0295
1714	M	31	7.9300	2.3830	3.9740	14.2870
1951	F	75	1.3400	0.1625	0.2495	1.7520
1952	F	63	26.4455	15.4145	10.0535	25.4680
1953	F	77	3.7025	1.4715	2.0715	7.2455
1954	F	59	0.5275	0.5315	0.3185	1.3775
1955	F	60	3.2500	1.2750	0.3620	4.8870
1956	F	48	0.7720	0.5685	0.3000	1.6405
1957	F	46	0.6405	0.6915	0.1765	1.5085
1958	F	58	1.7970	1.2220	0.2245	3.2435
1959	F	86	14.5210	25.2650	25.1265	64.9125
1960	M	21	0.2570	0.0715	0.0675	0.3960
1961	M	23	0.0190	0.1770	0.1540	0.3500
1962	M	23	BDL	0.0925	0.1025	0.1950
1963	M	57	88.3370	4.2590	2.0810	94.6770
1964	M	35	0.1095	0.1045	0.1215	0.3355
1965	M	57	89.9595	33.5045	38.9845	72.4890
1966	M	55	10.6895	14.1235	5.3605	30.1735
1967	M	14	1.0025	0.2110	0.1775	1.3910
1968	M	23	0.6025	0.7075	0.6005	1.9105
1969	M	45	0.7910	0.7590	0.1695	1.7195
1970	M	44	0.5115	1.3650	1.4290	3.3055

BDL, below detection limit ( $<0.01$  ng).

Table 5. Results from VX Cellophane Tape Strip Assays of PND 70 Rats

Rat No.	Sex	Dose (µg/kg)	VX from Tape Strip (ng)			Total VX (ng)
			A	B	C	
1715	F	78	68.2470	44.7811	13.2912	126.3194
1716	F	23	2.3380	2.6290	2.2880	7.2550
1717	F	75	5.0145	22.5125	7.5965	35.1235
1718	F	190	239.6187	84.8342	85.6728	410.1257
1720	M	114	20.2271	11.7680	5.8966	37.8917
1722	M	312	70.4687	93.0449	158.1307	321.6443
1723	M	48	100.5469	162.6624	50.9559	314.1652
1725	F	115	102.9345	34.8726	62.5585	200.3656
1726	F	308	565.0794	176.1469	134.8278	876.0541
1728	F	33	8.3730	6.8295	2.8205	18.0230
1729	F	57	5.9920	1.5010	0.7145	8.2075
1730	M	35	3.5670	0.7110	1.3830	5.6610
1731	M	314	722.7449	40.3690	124.2150	887.3289
1732	M	190	149.0844	115.4805	77.9948	342.5597
1733	M	21	0.0310	0.1860	0.9695	1.1865
1734	M	21	0.3575	0.0980	0.0225	0.4780
1735	F	33	6.1890	6.0785	4.7785	17.046
1737	F	57	11.8770	7.0015	4.8240	23.7025
1738	F	56	3.7340	1.5055	6.0125	11.2520
1740	M	187	283.7884	107.7844	52.1428	443.7157
1741	M	48	32.8240	1.9465	13.1050	47.8755
1742	M	35	6.0265	3.8230	4.1065	13.9560
1743	M	14	2.2980	2.2230	2.3375	6.8585
1744	M	110	152.9808	32.8491	37.4477	223.2776
1855	F	51	10.5252	5.1148	2.6683	18.3083
1856	F	54	45.7310	53.0821	36.5000	135.3131
1857	F	86	289.2550	48.4004	97.9539	435.5913
1858	F	93	272.1533	106.0051	49.3742	427.5326
1859	F	43	17.4757	80.9546	47.6330	146.0633
1860	F	87	143.5204	64.2519	22.2512	230.0235
1861	F	46	53.0149	70.5562	14.3188	137.8899
1862	F	56	187.1584	179.4451	88.4255	455.0290
1863	F	43	13.2516	15.2675	9.5840	38.1031
1864	M	73	14.8228	5.1625	9.8144	29.7997
1865	M	38	95.8440	81.6372	48.8796	226.3608
1866	M	45	34.5197	23.6253	27.2170	85.3620
1867	M	73	96.2128	42.5737	61.6391	200.4256
1868	M	37	10.6535	2.4767	0.9749	14.1051
1869	M	75	266.5629	70.4374	106.6514	443.6517
1870	M	51	158.6161	75.9905	104.4465	339.0531
1871	M	51	59.5254	73.6324	25.8164	158.9742
1872	M	43	19.4629	13.7715	1.5831	34.8175

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## ACRONYMS AND ABBREVIATIONS

ACh	acetylcholine
AChE	acetylcholinesterase
BDL	below detection limit
CAS	Chemical Abstracts Service
CASARM	chemical agent standard analytical reagent material
CE	collision energy
CI	chemical ionization
CWNA	chemical warfare nerve agent
ECBC	U.S. Army Edgewood Chemical Biological Center
ESI	electrospray ionization
GA	ethyl <i>N,N</i> -dimethylphosphoramidocyanidate; tabun
GB	isopropyl methylphosphonofluoridate; sarin
GC	gas chromatography
GD	pinacolyl methylphosphonofluoridate; soman
GF	cyclohexyl methylphosphonofluoridate; cyclosarin
IPA	2-propanol
IS	internal standard
KF	potassium fluoride
LC	liquid chromatography
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
<i>m/z</i>	mass-to-charge ratio
NB	laboratory notebook
NMR	nuclear magnetic resonance
NS	no sample received
PND	postnatal day
<i>R</i> <sup>2</sup>	coefficient of determination
Russian VX	<i>O</i> -isobutyl <i>S</i> -[(2-diethylamino)ethyl] methylphosphonothioate
SPE	solid-phase extraction
TEWL	transepidermal water loss
USAMRICD	U.S. Army Medical Research Institute of Chemical Defense
VX	<i>O</i> -ethyl <i>S</i> -(2-diisopropylaminoethyl) methylphosphonothioate
VX-G	<i>O</i> -ethyl methylphosphonofluoridate



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